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Abstract: Chronic viral infections and malignant tumours induce T cells that have a reduced ability to secrete effector cytokines and have upregulated expression of the inhibitory receptor PD1 (programmed cell death protein 1). These features have so far been considered to mark terminally differentiated 'exhausted' T cells. However, several recent clinical and experimental observations indicate that phenotypically exhausted T cells can still mediate a crucial level of pathogen or tumour control. In this Opinion article, we propose that the exhausted phenotype results from a differentiation process in which T cells stably adjust their effector capacity to the needs of chronic infection. We argue that this phenotype is optimized to cause minimal tissue damage while still mediating a critical level of pathogen control. In contrast to the presently held view of functional exhaustion, this new concept better reflects the pathophysiology and clinical manifestations of persisting infections, and it provides a rationale for emerging therapies that enhance T cell activity in chronic infection and cancer by blocking inhibitory receptors.

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T-cell differentiation in chronic infection and cancer – functional adaptation or exhaustion?

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Summary

Chronic viral infections and malignant tumors induce T-cells with a reduced ability to secrete effector cytokines and up-regulated PD-1 expression. These patterns have so far been considered to mark terminally differentiated “exhausted” T-cells. Several clinical and recent experimental observations, however, indicate that phenotypically “exhausted” T-cells still mediate a critical level of pathogen or tumor control. We are therefore reviewing evidence indicating that the exhausted phenotype results from a differentiation process in which T-cells stably adjust their effector capacity to the needs of chronic infection. We argue that this phenotype is optimized to cause minimal tissue damage while still mediating a critical level of pathogen control. In contrast to the presently held view of functional exhaustion, this new concept reflects better the pathophysiology and clinical manifestations of persisting infections and it provides a rationale for emerging therapies that enhance T-cell activity in chronic infection and cancer by blocking inhibitory receptors.

Main Text

1. Introduction

Most pathogens are rapidly cleared and the immune system becomes only transiently exposed to foreign antigens and inflammation. Such acute infections trigger the generation of large numbers of short-lived effector T-cells and the formation of memory T-cells¹⁻⁸. Following such infections, T-cells typically co-express multiple cytokines (TNF, IFN γ , IL-2) and cytotoxic effector molecules - a phenotype often referred to as polyfunctional⁹. A different outcome arises when infections become chronic and when viruses persist at high levels over long periods as in Human-Immunodeficiency-Virus (HIV) or Human-Hepatitis-C Virus (HCV) infections. Such conditions induce T-cells lacking the typical polyfunctional phenotype and T-cells retain the expression of inhibitory receptors including PD-1, Lag-3, Tim-3, and CD160⁹⁻¹⁷. Very similar phenotypes can be observed, when T-cells are long-term exposed to tumor-antigen¹⁸⁻²⁰. The prevailing view is that this phenotype marks functionally severely impaired so called “exhausted T-cells”, that conventional memory T-cells are not being formed in persisting infections²¹, and that chronic infections cause a progressively weakening response and a shutdown of T-cell mediated immunity.

However, several clinical observations and far reaching experimental evidence, including the therapeutic effects achieved by blocking PD-1 signaling, are difficult to align with the concept that the T-cell response only progressively deteriorates in chronic infections. Moreover, the term “exhausted T-cells” implies that T-cells acquire a non-functional status. However, we question whether the multifaceted expression pattern of receptors that inhibit T-cell function have solely evolved to render T-cells non-functional. Such an outcome could be achieved by simply deleting antigen-specific T-cells.

To resolve these discrepancies, we are proposing a significant revision of the current concept of T-cell responses in chronic infections. Following the idea that chronic infections require an equilibrium between control of viral replication and the level of immune-mediated pathology caused by the persisting T-cell responses²², we argue and provide evidence that functional adaptation, specialization, and the acquisition of stable phenotypes, rather than exhaustion, are the driving forces that determine T-cell response in chronic infections. Thus, we posit that the phenotype of exhausted T-cells actually reflects a finely tuned effector population that is optimized to fulfill a certain level of effector function and pathogen control without causing overwhelming immunopathology.

2. Origin of the T-cell exhaustion concept

The idea that chronic infections exhaust the T-cell response was originally formulated following studies in mice persistently infected with the *Lymphocytic choriomeningitis virus* (LCMV) docile strain. In these mice, the possibility to *in vitro* expand antigen-specific T-cells declines over time which led to the conclusion that a continuing infection “exhausts” or severely diminishes the pathogen-specific T-cell response¹³. The introduction of the tetramer technology and transgenic CD8 T-cells specific for an LCMV-derived antigen (P14) enabled more detailed characterizations and proved a substantial loss of effector T-cells. Nonetheless, depending on the pathogen dose and the epitope being studied, a significant fraction of T-cells bypasses deletion. This applies in LCMV clone-13 infections to T-cells specific for the gp33 peptide while their np396-specific counterparts become deleted^{12,14}. The latter is thought to result from a higher avidity of T-cells for the np396 than for the gp33 peptide²³. Moreover, the availability of LCMV strains which differ in their *in vivo* persistence (i.e. acute WE or Armstrong and chronic clone-13 or docile strains) facilitated in depth characterizations of how the duration of infections caused by very similar pathogens impacts T-cell differentiation. These studies prompted the discovery of the abovementioned chronic infection phenotype^{15,24} and established that acquiring

this phenotype and deletion of antigen-specific T-cells are in parallel or sequentially occurring events. Taking into count that T-cells which do not become deleted show a different phenotype, that they are difficult to re-expand *in vitro*, and that they do not survive well following adoptive transfer into antigen negative hosts²⁵ all this sustained the concept that the residual T-cells are non-functional - a notion that intuitively goes along with the inability of the immune-system to clear the virus. Alongside, the use of the term “T-cell exhaustion” significantly evolved - from referring to the elimination of antigen-specific T-cells to now being predominantly used to refer to T-cells which bear the chronic infection phenotype. While antigen-specific T-cells in persisting infections clearly differ from their counterparts found in acute infections, we focus our review on aspects highlighting that these retained T-cells are not an inert non-functional population and we favor the view that these retained T-cells fulfill special functions. We are thereby also resolving the confusion that we refer to these T-cells as being exhausted despite the strong evidence that we can re-activate their effector potential for instance by blocking PD-1 signaling.

3. Evidence that functional T-cells are maintained in chronic infections and tumors

A characteristic feature of many persisting infections, including HIV, SIV [Simian Immunodeficiency Virus] or HCV infections, is the appearance of virus variants expressing mutated T-cell epitopes. This phenomenon clearly requires ongoing antigen-specific CD8⁺ T-cell function but whether or not this selection can only be mediated by T-cells with a normal effector phenotype or how much T-cells with an exhausted phenotype could be involved remains unclear. Nonetheless, the late occurring appearance of mutant viruses in established chronic infection is a fundamental indicator for long-term maintenance of functional T-cell responses and highlights that exhausted T-cell populations possibly contribute to select escape variants^{26,27}. The association between the late emergence of escape mutations, concomitant steep viral load increase, and disease progression to AIDS in HIV infections underline the aforementioned considerations. Moreover, the numbers of HIV-specific T-cells can rise following

an interruption of anti-retroviral therapy^{28,29}. Along with the evidence that HIV infections impair the thymic output, this furthermore reveals the retention of T-cells in established chronic infections that can expand substantially^{30,31}. The demonstration that virus titers strongly rise following depletion of CD8⁺ T-cells in SIV infected macaques³²⁻³⁴ and that protective immune responses can be elicited upon vaccinating SIV infected Rhesus macaques³⁵ further underscore the strong effector capacity of CD8⁺ T-cells in established chronic infections.

T-cells with an exhausted phenotype can also be found following chronic antigen stimulation in tumors^{19,36-38}. Despite showing this phenotype, there is a clear positive correlation between number of CD8⁺ tumor infiltrating lymphocytes (TIL) and a favorable clinical prognosis^{39,40}. Remarkably, 58 out of 60 studies highlight this correlation across patients with all major types of cancer³⁹⁻⁴¹. Consistent with the aforementioned immune control in the presence of phenotypically exhausted T-cells, there is evidence that PD-1^{high} CD8⁺ T-cells can express similar or even elevated levels of cytotoxic molecules compared to cells with a less pronounced exhausted phenotype^{37,42-45}.

The strongest evidence that exhausted cells retain the ability to mount a potent effector response comes from the many studies in which strong anti-tumor or anti-viral immunity could be restored by blocking PD-1/PD-L1 interaction alone or in combination with targeting other receptors⁴⁶⁻⁵². This approach of antibody-mediated “checkpoint blockade” is now increasingly used to treat patients with cancer and its impact on chronic infections in humans is being evaluated (Box 1). The efficacy of blocking PD-1 signaling strongly implies that T-cell function in chronic infections and cancer is not solely attenuated because of a CD8 T-cell intrinsic loss of function but rather because the function of these T-cells is controlled by external regulatory mechanisms that involve PD-1 signaling.

Another point to consider is that LCMV clone-13 infections, in which many features of exhausted T-cells including the relevance of PD-1 expression were established^{15,46}, show virus load kinetics that are most indicative for a long-term maintained effector T-cell response. Starting from high levels, the virus titer usually strongly declines to levels that are undetectable in the blood within 4-8 weeks post infection and also decreases in infected organs with the kidneys being a major exception as they continue to contain high titers^{14,54}. Though the precise protective mechanisms underlying the decline remain unclear, this observation signals that the presence of an exhausted T-cell phenotype does not preclude substantial virus control.

Infections which show a latent phase (for example Epstein-Barr-Virus [EBV] or Cytomegalovirus [CMV] infections) differ in many respects from the aforementioned persisting infections but they are similar in such that T-cells become repetitively exposed to antigen. The mostly undetectable pathogen loads and the occasionally occurring virus re-activation imply that T-cells are exposed only to low antigen levels. Nonetheless, T-cells specific for antigens derived from latently-persisting pathogens often increase over time - a process known as memory inflation⁵⁵. T-cells with such specificity often appear more activated than memory T-cells formed following resolved infections and they express KLRG-1⁵⁶ – a marker that is typically expressed by T-cells in the acute infection phase²⁴. The latter indicates substantial antigen exposure over time and yet the T-cells retain a high functional capacity and strongly respond to virus re-activation⁵⁷⁻⁵⁹. Thus, repetitive exposure to pathogen-derived antigen does not preclude or extinct functional effector and memory T-cell responses. Irrespectively of the repetitive antigen exposure, T-cells specific for latent infections lack or express only very low levels of PD-1 indicating that PD-1 might in these infections signal recent activation⁶⁰. The latter also suggests that stable PD-1 expression might strictly correlate with the strength of antigen-stimulation, which is possibly lower in latent than in active chronic infections. This idea would be well in line with the concept that PD-1 serves as a rheostat following strong T-cell stimulation⁶¹. Moreover, several studies in active

chronic infections established links between the magnitude of T-cell stimulation (or TCR avidity) and T-cell fate. Accordingly, lower avidity or weaker stimulated T-cells are more prone to retain a normal phenotype while higher avidity T-cells acquire a chronic infection phenotype or become deleted^{62,63}. This is somewhat reminiscent of the fact that T-cells retain a rather normal effector phenotype in latent infections in which T-cells most likely receive lower TCR stimulation due to a lower level of antigen-exposure.

4. T-cells undergo stable differentiation in persisting infections and form memory-like cells

The aforementioned examples underline that CD8⁺ T-cells retain at least partial control over virus load in chronic infections and tumors at time points, when the majority of T-cells show an “exhausted” phenotype. What remains unclear is how such long-term persisting T-cell responses are maintained, which fraction of cells remain functional and what degree of virus control can be mediated by T-cells in chronic infections. To address this, we recently asked what happens when phenotypically “exhausted” T-cell populations are removed from a chronic LCMV clone-13 infection and re-exposed to antigen in the context of an acute LCMV Armstrong infection⁶⁴. Surprisingly, we observed that T-cells which homogenously expressed high levels of PD-1 and low levels of cytokines can undergo robust re-expansion, can form secondary effector T-cells, and even protected against pathogen challenge following transfer into acute infections.

Unexpectedly, even though the transferred cells were exposed only to an acute infection, the vast majority of re-expanded T-cells still displayed the “exhausted” T-cell phenotype as they continued to express low levels of cytokines and retained PD-1 expression. Most interestingly, the “exhausted” phenotype was retained even when T-cells from chronic infection were challenged 4 weeks after they had been transferred into naïve hosts (Figure 1). This outcome also implies the presence of T-cells with memory-like properties among the T-cells transferred into the naïve hosts – a notion that will be discussed in more detail below⁶⁴. This stability of the exhausted phenotype is supported by epigenetic studies showing that chronic LCMV clone-13

infections induce profound and permanent de-methylations in the PD-1 promoter region. The de-methylation was maintained in antigen-specific T-cells long after LCMV clone-13 virus load has dropped below detectable levels in the blood⁶⁵. A lack of methylation in the PD-1 promoter region has also been observed among antigen-specific CD8 T-cells from HIV patients which have been treated long-term (>2 years) with anti-retroviral therapy or in elite controllers⁶⁶. The latter further indicates a fixation of the epigenetic imprint in the PD-1 locus. The view that T-cells undergo a stable form of differentiation in chronic infections is also supported by studies showing that in spontaneously resolved HCV infection, CD8⁺ T-cells retain an exhausted phenotype even after pathogen clearance⁶⁷.

What remains unknown, however, is when T-cells undergo this differentiation. The incremental appearance of T-cells with an “exhausted” phenotype led to the conclusion that prolonged exposure to antigen and inflammation causes a progressively occurring acquisition of an exhausted phenotype⁶⁸. Of note, this progression has only been described at the level of the entire population of antigen-specific T-cells but a direct demonstration that a T-cell, which at an early phase of a chronic infection displayed a normal phenotype, shows an exhausted phenotype at a later stage has not yet been performed. It also needs to be acknowledged that a fraction of virus-specific T-cells show already in the early phase of a LCMV clone-13 infection the typical mono-functional cytokine signature (low level IFN γ , no TNF production) and that a considerable number of antigen-specific T-cells display this phenotype in the early phase of acute infections²⁴. This suggests that T-cells displaying a key feature of “exhaustion” might be constitutively generated following activation in viral infections. Thus, T-cells with a normal profile and T-cells with an “exhausted” phenotype might be formed in parallel in the early phase of an infection and, depending on whether or not the infection remains acute or becomes chronic, a selective outgrowth occurs over time favoring one over the other T-cell phenotype. This concept that the “exhausted” T-cell phenotype could be generated already at very early stages during

the infection and that these T-cells progressively increase - from an initially numerically underrepresented population to becoming a dominant population - would also be very well in line with the aforementioned phenotypic stability of the “exhausted” phenotype.

Two recent papers resolved the bulk population of antigen-specific T-cells in acute infections into cells that originate from the same naïve precursor (referred to as T-cell families)^{69,70}. Interestingly, families of T-cells that are initially underrepresented at the population level and whose phenotype and cytokine secretion profiles substantially differed from the average phenotype of the population, showed the highest secondary re-expansion potential. Thus, a higher re-expansion potential of underrepresented populations or families could effectively reshape the global phenotype of a T-cell population following re-expansion. Such remodeling could also occur over time in chronic infection and this could result in a preferential outgrowth or selective survival of T-cells stably displaying an exhausted phenotype over T-cells that show a conventional phenotype and which dominate the early phase of chronic infection.

5. Why are memory-like cells formed in chronic infections?

The aforementioned survival of a fraction of exhausted T-cells following transfer into antigen-free hosts, the ability of these T-cells to undergo re-expansion, and that the cells retain a particular phenotype are hallmark features of memory T-cells⁶⁴. We therefore propose that a fraction of T-cells in chronic infections form cells that behave like memory cells and that pass an exhausted phenotype to their progeny (Figure 2). Memory T-cells formed after an acute infection ensure a rapid generation of large numbers of effector T-cells upon pathogen re-exposure^{5,6,71}. But why should cells with memory-like behavior be formed in chronic infections? These cells could in chronic infections constitute a reservoir to sustain the pool of effector T-cells (Figure 2). In fact, it appears very unlikely that effector T-cells, which are formed for instance at the beginning of an HIV or HCV infection, may persist over months or years. More

likely, these effector populations are maintained through memory-like cells that constantly replenish a pool of otherwise short-lived effector T-cells. Such a replacement seems to occur in CMV infections⁵⁵. A precursor progeny relation between subpopulations of T-cells that differ in expressing Tbox transcription factors has also been shown in LCMV clone-13 infected mice⁷². This strongly suggests that long-term effector T-cell responses in chronic infections are maintained by both functional diversification and cells with memory-like features.

Interestingly, it has long ago been realized that the elimination of CD4 T-cells increases total virus load and decreases the number of pathogen-specific T-cells in chronic LCMV infections^{14,54}. The precise mechanisms how CD4 T-cells enhance survival and effector function of CD8 T-cells remain unclear. We hypothesize that CD4 T-cells might fulfill in chronic infections a similar function in maintaining memory T-cells as following acute infections⁷³. Accordingly, the deterioration in the T-cell response following CD4 depletion in chronic infection might be a consequence of losing these memory-like T-cells. Interestingly, a recent publication indicated that the impact of CD4 T-cells on CD8 T-cells in chronic infection could also be controlled by NK cells, which reduce the number of CD4 T-cells and concomitantly the number of CD8 T-cells⁷⁴.

We consider that memory-like T-cells comprise only a very small fraction of the population of antigen-specific T-cells in chronic infections. This would be well in line with the rapid and more than 90% decline of engrafted T-cells observed when T-cells from clone-13 infected mice were transferred into naïve mice in the absence of pathogen re-exposure^{21,25,75}. We argue that this decline reflects only the loss of effector-like T-cells but it does not preclude the selective survival of a few memory-like T-cells. Besides, a decline of similar magnitude and kinetics occurs when day 8 effector T-cells from acute infections are transferred into naïve mice and yet a low number of memory T-cells survive⁷¹. Moreover, the notion that the bulk population of phenotypically exhausted T-cells is gene expression wise related to effector and not to memory cells does also not contradict our proposed model⁷⁶. Instead, it rather means that specific-gene expression

patterns of the small sub-fraction of memory-like T-cells are most likely diluted by the numerical dominance of effector-like cells in the population of phenotypically exhausted T-cells.

6. PD-1 and other inhibitory receptors enable a functional control of effector T-cells

The goal of an immune response is to remove a pathogen. To achieve this, aggressive mechanisms are engaged at the risk of collateral tissue damage. We consider that the immune system adopts a different strategy in persisting infections that is driven by the need to balance pathogen control and tissue damage²². So far, the reduced ability of T-cells to produce cytokines in chronic infections has been seen as a sign of deteriorating T-cell function but this phenotype could also simply represent an effector stage that is optimized to cause less inflammation and immunopathology than normal effector T-cells. Such an adjustment in combination with persisting expression of PD-1 could be of vital importance in chronic infections^{64,65}. In strong support of this concept, it was recently shown that a high dose virus infection, which induced a strong “exhausted” phenotype, protected mice from immunopathology while a lower dose, which failed to induce an “exhausted” phenotype, resulted in substantial pathology^{74,77}. Similarly, disrupting the von Hippel-Lindau tumor suppressor (VHL) gene causes overwhelming immunopathology by enforcing cytotoxic activity of T-cells in persisting infections⁷⁸.

PD-1 down-modulates T-cell function in chronic infections but possibly also in acute infection as PD-1 is readily up-regulated following T-cell activation²⁴. It also needs to be considered that it can be difficult to distinguish PD-1 expression resulting from recent stimulation versus the maintained expression seen in chronic infection. What remains however unclear in chronic infections is when and how the PD-1 pathway is utilized. The sustained virus control in chronic infections argues against constitutive PD-1/PD-L1 interaction. Perhaps, PD-1 and other inhibitory pathways are primarily acting to prevent T-cell responses in particular circumstances

and against tissues essential for the survival of the host. In line with this, differences in the expression of PD-1 and its ligands in different tissues have been described⁴². Moreover, a critical role of PD-1/PD-L1 mediated protection from endothelial damage and an otherwise resulting circulation failure has recently been demonstrated⁷⁹. Fixing PD-1 expression in T-cells in chronic infection would ensure that this axis remains accessible, always ready to act as a potent control mechanism to limit T-cell mediated tissue damage⁸⁰. Interestingly, tumor cells appear to benefit from this pathway and shield themselves against cytotoxic responses by expressing PD-1 ligands⁸¹⁻⁸³.

An important question is why blocking PD-1 in an established chronic LCMV clone-13 infection does not cause severe tissue damage. While this remains to be further investigated, it could be a question of timing but also efficacy of inhibition. Indeed, blocking PD-1/PD-L1 interaction too early in LCMV clone-13 infections strongly increases the severity of immunopathology⁷⁹. This could be seen as a further indication that in late-stage chronic infections T-cells underwent functional specialization and cause, even when they are unleashed from PD-1/PD-L1 mediated inhibition, significantly less immunopathology than more aggressive early-stage effector T-cells. Stable PD-1 expression could also be very important to functionally control memory T-cells residing in tissues vulnerable to viral re-infection. The concept of tissue-resident memory CD8⁺ T-cells is now established for several types of tissues and infections⁸⁴⁻⁸⁷. Indeed, PD-1 positive CD8⁺ T-cells have been found to be preferentially retained in bone marrow, lymph nodes, kidney, and brain after chronic LCMV clone-13 infection⁴². Moreover, brain-resident effector memory CD8⁺ T-cell populations after Vesicular Stomatitis Virus infection in mice were found to express high levels of PD-1 and CTLA-4⁸⁸. At the same time, these resident T-cells often contain high levels of granzymes⁸⁹. Thus, viral infections establish resident memory T-cell populations in organs repetitively targeted by similar pathogens to enable rapid pathogen control. The presence of PD-1 on such resident-memory cells suggests a need to safeguard the

cytotoxic and immunopathology causing potential of these cells by functionally controlling their effector function through the ligation of inhibitory receptors.

7. Overall perspective

In contrast to the idea of a sole deteriorating T-cell response in persisting infections, we argue in favor of the existence of a prolonged effector phase in chronic infection during which significant virus control is maintained by T-cells well adapted and specialized to the conditions of chronic infections. We consider that these T-cell responses are maintained through the formation of memory-like cells that stably transmit an exhausted phenotype to otherwise short-lived effector T-cells. In our opinion, such a prolonged effector phase reflects much better the physiology of chronic infections. Instead of using the term “exhausted” we therefore suggest using the term chronically activated effector T-cells following the proposed concept that these T-cells retain a certain level of effector function. Nonetheless, more detailed studies are needed to compare their protective capacity with that of normal effector T-cells.

We anticipate that this prolonged effector phase only lasts for a certain period and that there is a final late stage at which a decline in the numbers of these chronically activated effector T-cells and their corresponding memory-like counterparts occurs, ultimately leading to the extinction of a functional T-cell response (Figure 2). The latter view is well in line with observations that T-cell responses in chronic HCV or HIV/SIV clearly decline when analyzed over long periods and also with observations indicating that even cells generated in acute infections show a decreasing ability to respond after going through multiple rounds of re-stimulation^{90,91}. The length of this prolonged effector phase during which chronically activated effector T-cells retain considerable control over virus and tumor burden may vary between individuals and different types of infection. Presently, the major challenge is therefore to find ways to maintain T-cells in this

prolonged effector phase, to prevent their deterioration, and to increase their function beyond what can be achieved with “checkpoint blockade”.

Text Box

Clinical evidence that blocking inhibitory receptors expressed on tumor- or virus-specific T-cells enhances anti-cancer immunity and virus control.

Malignant diseases:

Clinical trials in which significant clinical benefits were observed upon injecting monoclonal antibodies specific for inhibitory receptors (a therapy known as “checkpoint blockade”).

- Ipilimumab (a monoclonal antibody) blocks CTLA-4 and improves disease outcome in patients with malignant melanoma⁹². This treatment has been approved by FDA and EMA in 2011.
- Promising clinical results were obtained by blocking PD-1/PD-L1 interactions in patients with melanoma, lung and kidney cancer^{82,93-95}. Recent data suggest that this approach may be more efficient and less toxic as compared to anti-CTLA-4 treatment.
- Two phase 1 clinical trials also suggested that combined PD-1 and CTLA-4 blockade could further improve the clinical responses (but with increased toxicity) in melanoma patients⁹⁵⁻⁹⁷.
- Currently, the reference webpage for clinical trials (www.clinicaltrials.gov) lists 320 trials in which inhibitory receptors are targeted for treating cancer patients (anti-CTLA-4: 216 trials; anti-PD-1: 65 trials; anti-PD-L1: 33 trials; anti-PD-L2: 1 trial; anti-LAG-3: 2 trials; anti-KIR (Killer-cell Immunoglobulin-like Receptor): 3 trials)

These trials are based on numerous research studies (reviewed in references^{18,98}).

Infectious diseases:

Presently, the extent to which “checkpoint blockade” improves the clinical manifestation of persisting infections is being evaluated. Compared to the tumor field, much less information is available concerning its efficacy in chronic infections but several promising observations have been made some of which are listed below. Nonetheless, more clinical studies are needed to sustain these early observations:

- A single dose of injecting PD-1 blocking antibody decreased viral titers in 11-15% of HCV patients. This included non-responders to the standard interferon therapy⁹⁹.*
- Treatment of HCV infected chimpanzees with PD-1 blocking antibodies enhanced anti-viral T-cell responses and reduced viremia¹⁰⁰.*
- In-vivo manipulation of PD-1/PD-L1 interactions was shown to enhance CD8⁺ T-cell functions and extend the survival of SIV infected nonhuman primates¹⁰¹.
- In vitro studies showed that the cytotoxic ability of HIV specific T-cells could be rescued by blocking Tim-3⁴⁵.
- Recently, a melanoma patient was treated with anti-CTLA-4 mAb. Incidentally, his HCV infection was successfully controlled, despite that the patient did not receive anti-viral therapy¹⁰².
- Currently, there are 4 ongoing trials in which anti-PD-1 or anti-PD-L1 antibodies are used to treat patients with chronic infectious diseases (www.clinicaltrials.gov).

* These are interesting initial observations albeit the overall patient benefit observed in these studies remained relatively low.

Figure legends

Figure 1: Assessing T-cell differentiation in acute and chronic infections

LCMV-specific CD8⁺ T-cells from acutely LCMV Armstrong or chronically LCMV clone-13 infected mice were transferred into naïve hosts. The cells were harvested from mice that had at least been infected 4 weeks earlier. Following the transfer, no spontaneous LCMV response was mounted by host CD8⁺ T-cells, which indicated that virus has not been transferred. 4 weeks after the transfer, both groups of secondary host mice were infected with an acute LCMV Armstrong infection. The exhausted T-cell phenotype acquired during the chronic infection (highlighted in red) could still be detected after the massive secondary expansion.⁶⁴

Figure 2: Proposed model for T-cell responses in chronic infection

Top: In resolved infections, short-lived effector T-cells vanish shortly after pathogen clearance while a long-term stable memory T-cell population forms. **Bottom:** We propose that also in chronic infections a population of memory-like T-cells forms; i) which shares key features with a conventional memory T-cell population; ii) whose function is to constantly replace the pool of otherwise short-lived effector T-cells and iii) which stably transmits the “exhausted phenotype” onto its decedents. This concept also suggests that the presence of a short-lived effector T-cell population is a shared property of acute and chronic infection with the difference that this population vanishes fast in acute infection while it is maintained by continuous replenishment over a long period (prolonged effector phase) in chronic infections. In line with clinical observations, we consider that this maintenance of the effector pool may eventually decline over time ultimately resulting in a failure to sustain the effector response.

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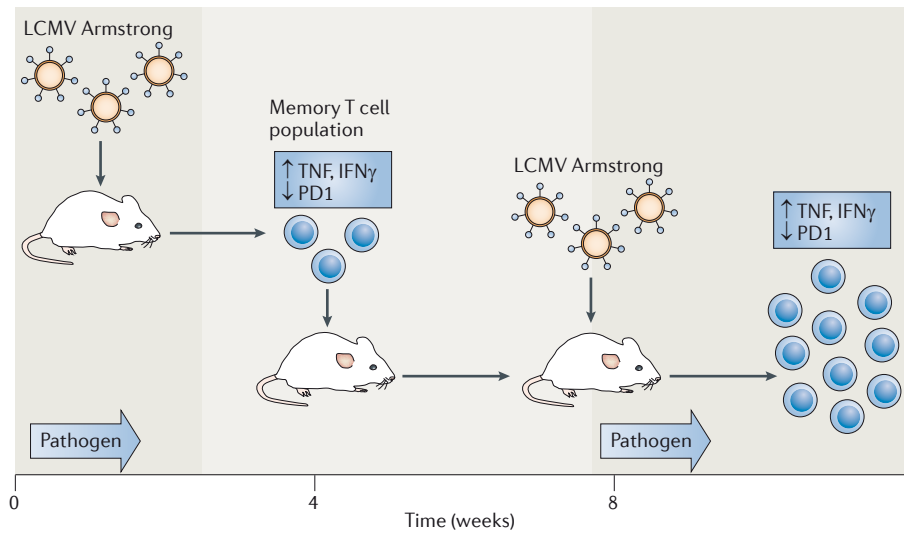
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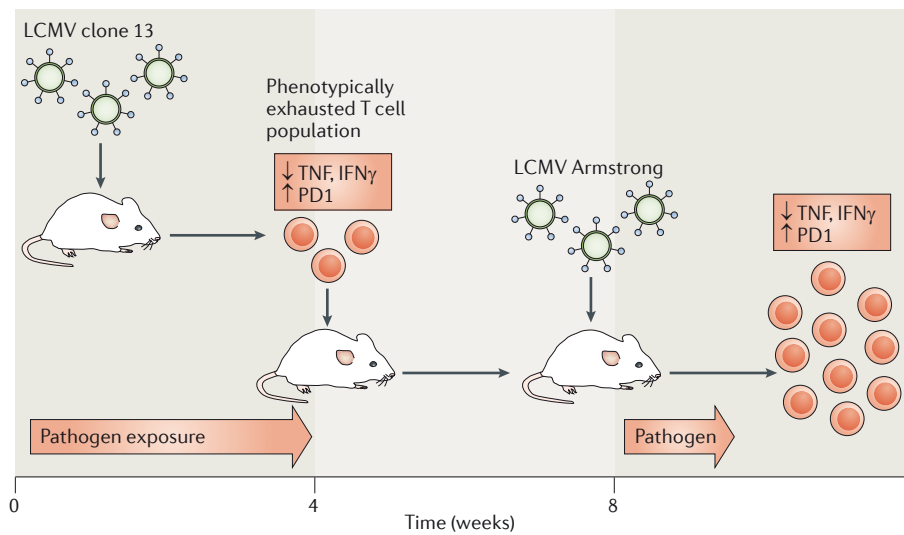
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Figure 1

a Acute infection



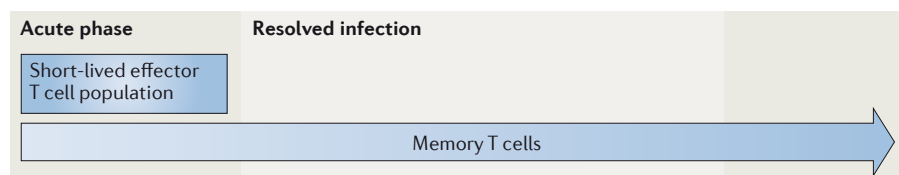
b Chronic infection



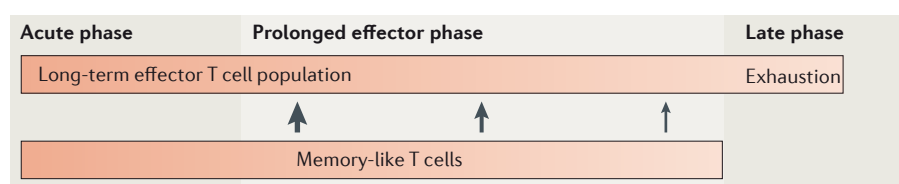
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Figure 2

a Resolved infection



b Persistent infection



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